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<p>14. ABSTRACT The central goal of these studies has been the integration of organic and inorganic components into well-defined structures using chemically functionalized viral capsids. Key strategies that have been explored include the use of new bioconjugation reactions to modify previously inaccessible sites on the protein surfaces and the preparation of water-soluble gold particles bearing reactive groups for biomolecules attachment. We have also developed a method for the attachment to viral capsids to carbon nanotubes using a new class of polymer-based surfactants.</p>				
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Virus-Based Scaffolds for Organic/Inorganic Hybrid Materials

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Project Summary, 2006

Introduction. The central goal of these studies has been the integration of organic and inorganic components into well-defined structures using chemically functionalized viral capsids. Key strategies that have been explored include the use of new bioconjugation reactions to modify previously inaccessible sites on the protein surfaces and the preparation of water-soluble gold particles bearing reactive groups for biomolecule attachment. We have also developed a method for the attachment to viral capsids to carbon nanotubes using a new class of polymer-based surfactants.

Preparation of hybrid materials through the dual surface modification of bacteriophage MS2. Task one focused on the development of synthetic techniques for the preparation of shell-like materials with differentiated interior and exterior surfaces. Specifically, we have developed a series of reactions that can attach inorganic particles to the exterior surface of a spherical virus after installing fluorescent chromophores inside. In these systems, the distance between the metal surface and the dye molecules is held fixed by the thickness of the protein shell, potentially allowing enhancement of the absorption characteristics of the interior dyes while preventing contact quenching.

The basic scaffold used for these studies is bacteriophage MS2, which consists of a hollow protein shell consisting of 180 identical protein building blocks, Figure 1a. Although these viruses initially possess a genome of 3800 RNA nucleotides, we have found in previous studies that the nucleic acids can be removed by exposing the particles to strongly basic conditions. The resulting “empty” genome-free capsids provide 27 nm shells that are highly stable in aqueous media from pH 3-10 and up to 60 °C. Of particular interest for interior modification is the presence of 32 pores that allow access to species smaller than 2 nm without requiring capsid disassembly.

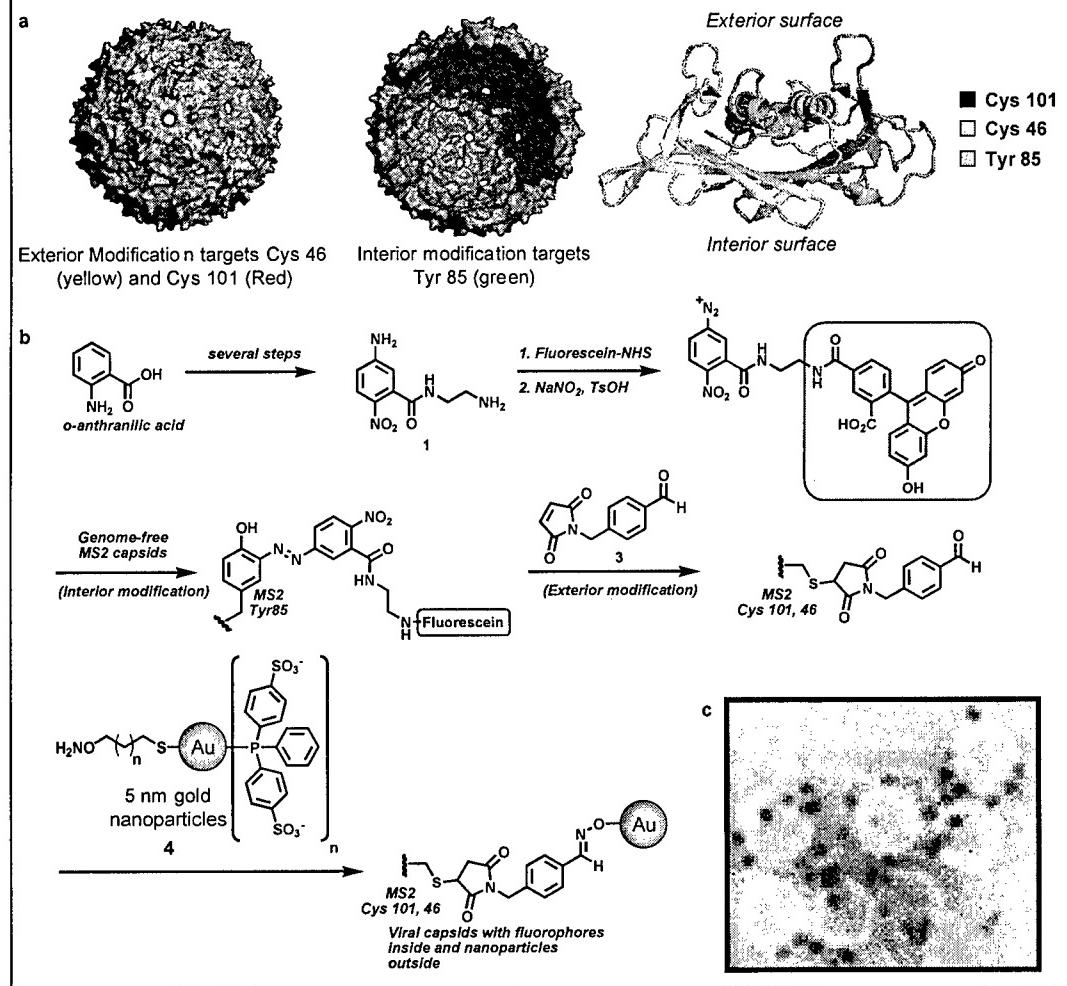
To install fluorescent molecules on the inside surface, we targeted 180 copies of a tyrosine residue (Tyr 85) using a diazonium coupling strategy previously described by our lab. Although our former studies have indicated that this site can be targeted in high yield and with virtually complete specificity, these experiments began with the synthesis of aniline **1** (through 6 steps) to allow the fluorophore attachment, Figure 1b. This compound can be attached to any commercially available NHS ester, including fluorescein-NHS. The resulting conjugate was converted to the diazonium salt (**2**) and coupled to the interior of MS2. Absorbance measurements indicated that 50-100 copies of the dye molecule were installed on inside each capsid.

The exterior surface of the MS2 capsids was decorated with 5 nm gold nanoparticles using a two step procedure. The exterior surface of each monomer presents 2 cysteine residues that can be functionalized using maleimides, such as reagent **3**. Functionalization with this reagent placed up to 360 aldehyde groups on the outside of the capsids, allowing further derivitization through carbonyl-selective reactions.

In parallel, we have synthesized gold nanoparticles that bear alkoxyamine groups designed to couple to the aldehydes introduced on the capsid surfaces. We have done this by preparing our own particles in addition to using commercially-available materials. After exchanging the initial passivating agents with triphenylphosphine-bis-sulfonate, bifunctional alkoxyamine-thiolates prepared in our lab were attached to the surface. The resulting particles (**4**) are stable in aqueous

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Figure 1. Preparation of hybrid organic/inorganic structures through the dual Surface modification of bacteriophage MS2.



solution under low ionic strength conditions and can be coupled readily to aldehydes and ketones introduced on biomolecule surfaces. In that case of aldehyde-functionalized MS2, several particles could be attached to the surface of each capsid, Figure 1c. Presumably complete coverage was not observed due to the building charge repulsion between the sulfonate groups on the particles. Nonetheless, a reasonable number of nanocrystals can be placed within 4 nm of the fluorescent molecules inside the capsids. Current efforts are evaluating the ability of the metal surfaces to enhance the spectral characteristics of the dyes.

The interaction of nanoscale materials with living cells is a frontier research area. With the fluorescent molecules installed inside our capsids, we took the opportunity to examine the uptake of MS2 by a number of mammalian cell lines, Figure 2a. Interestingly, two cell lines, C57MG (mouse breast cancer) and KB (human nasal-pharyngeal cancer), showed significant uptake of the capsids, while many other cell lines did not. Fluorescence microscopy analysis revealed punctuate fluorescence, which suggests uptake through an endosomal pathway, Figure 2b. In experiments using capsids bearing external polymers or biotin groups, no uptake was observed. This observation demonstrates the clear advantage of installing functionality inside the capsids to

avoid interference with cell targeting strategies. The nature of these uptake mechanisms is being elucidated in studies that are outside the scope of this program.

Attachment of gold particles to the surface of the tobacco mosaic virus. In parallel to the studies with bacteriophage MS2, we have carried out a number of synthetic studies based on the rod-like capsid of the tobacco mosaic virus. In this case, 2100 identical protein monomers self-assemble to form a hollow tube that is 300 nm in length. In previous studies, we have found that a tyrosine residue (Tyr 139) can be modified using diazonium chemistry, allowing thousands of functional groups to be installed on the outside surface of the capsids, Figure 3a. The spacing of these sites is 3.1 nm.

Using the same alkoxyamine-coated particles (4) described for the modification of bacteriophage MS2, TMV rods could also be coated with gold nanoparticles, Figure 3b. More complete surface coverage was observed in this case, although vacant sites were still prevalent in these structures as well. As this is believed to be due to charge repulsion between the anionic phosphine groups, we are currently preparing water soluble particles that are uncharged. This requires the synthesis of polyhydroxylated phosphines, a project that is currently underway.

In terms of other electroactive groups, we have also synthesized PEG polymers (5) that can be attached to the ketone groups on the TMV capsids, Figure 3c. These polymer chains possess pyrene groups, which cause the TMV rods to associate with carbon nanotubes. At low concentrations, linear TMV arrays are produced (Figure 3d), while at high concentrations, large mat-like materials result (Figure 3e). Electron microscopy has shown full integration between these materials of unlike solubility, suggesting that through the use of additional functionalization reactions TMV capsids could serve to incorporate many different components into carbon nanotube-based materials. Studies examining the effects of TMV attachment on the conductive properties of the tubes are in progress.

In a related research project, we have prepared a mutant form of the TMV coat protein that bears a reactive cysteine residue, Figure 4. We have found that this site can be modified with chromophore-maleimide reagents, and that the functionalized proteins can still assemble into helical structures. Related studies supported by LBNL and the DOE have determined that efficient light harvesting can be achieved using these materials.

Figure 2. Cellular uptake of fluorescently labeled MS2 viral capsids.

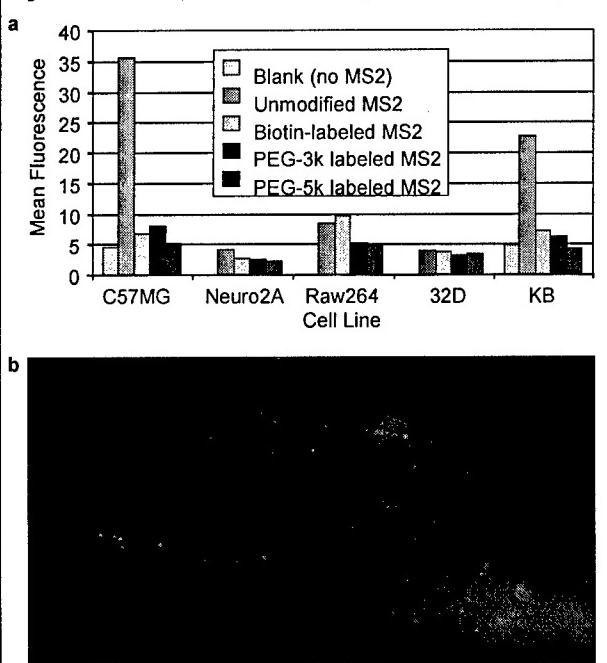
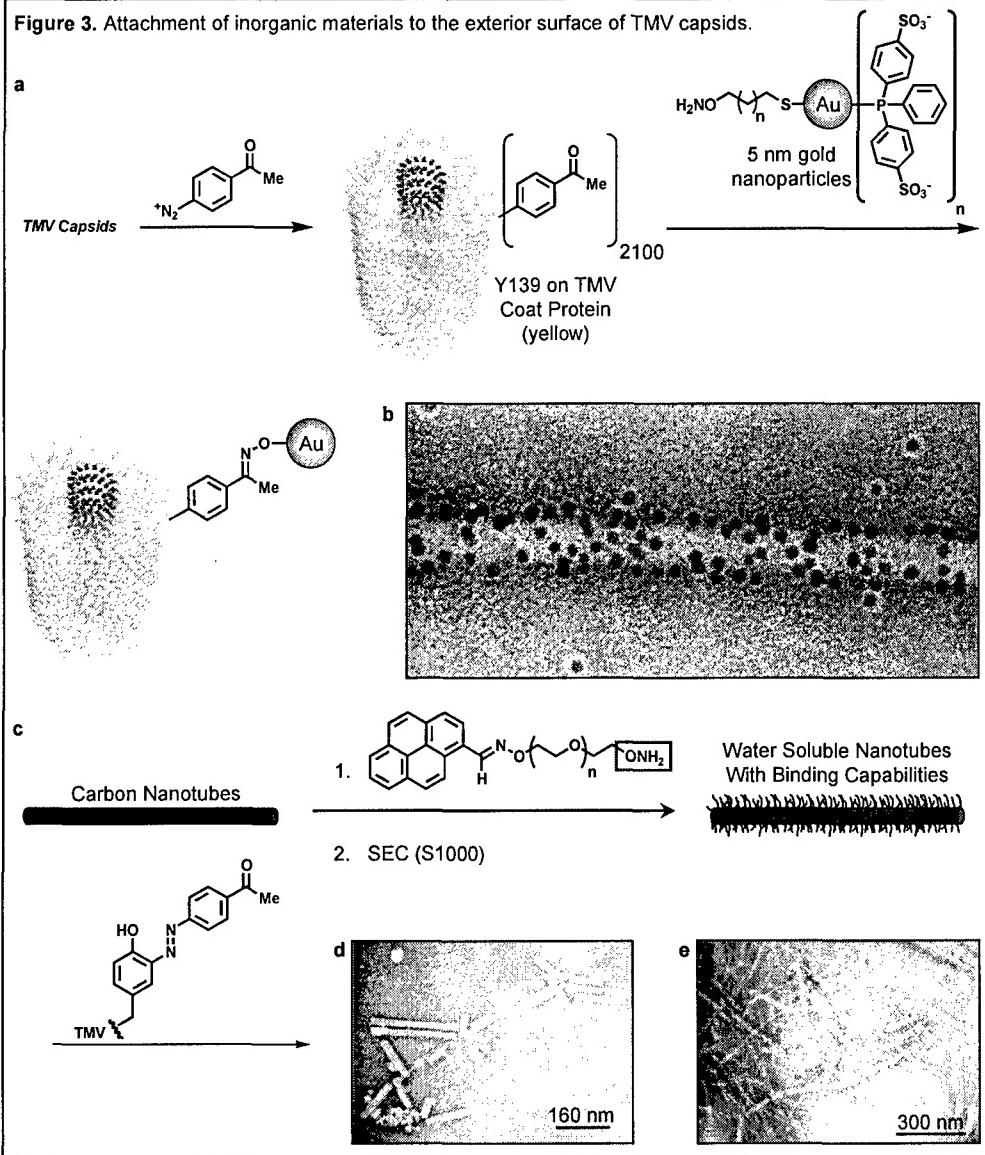


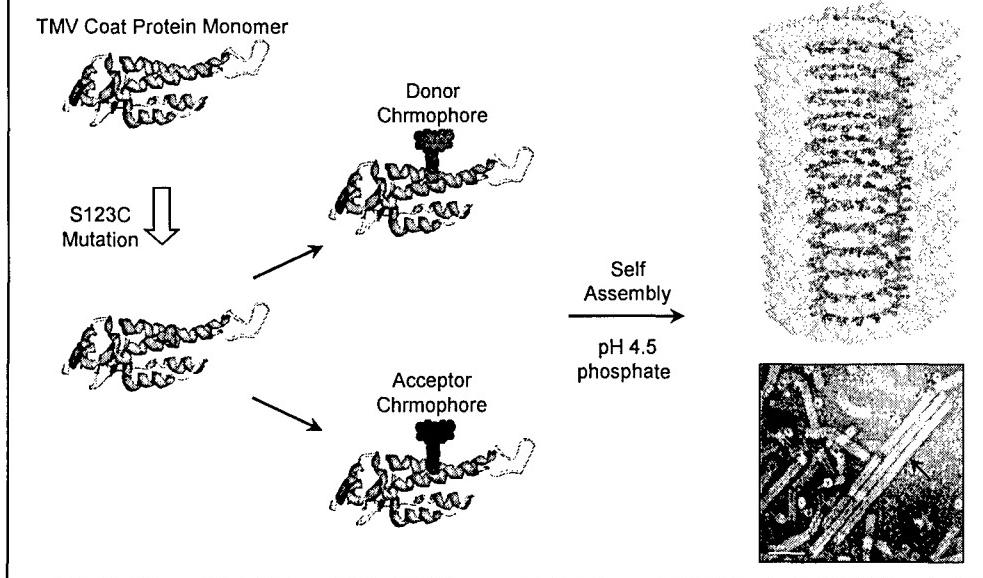
Figure 3. Attachment of inorganic materials to the exterior surface of TMV capsids.



However, the diazonium salt modification strategy used to attach gold nanoparticles to TMV rods is incompatible with these assemblies because they are unstable at the high pH conditions that this reaction requires. To address this problem, we are now preparing TMV monomers that can be attached both to chromophores and to nanoparticles in the same assemblies. The overall strategy involves the conversion of the N-terminus to a ketone group using an oxidative transamination reaction recently published by our group.

Conclusion. These studies have resulted in a significant increase in the range of TMV-based materials that can be prepared. Although most of the effort over the first year was focused on synthesis, we are now well-poised to characterize the optical properties of these structures. Efforts to increase nanoparticle coverage are also ongoing, as is a further exploration of TMV-carbon nanotube composite materials.

Figure 4. Preparation of organized chromophore arrays from synthetically modified TMV capsids.



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Amish Patel: (100%) synthesis of inorganic particles and attachment to viral capsid scaffolds

Patrick Holder: (25%) Synthesis of dual-functionalized polymers for attachment of viral capsids to carbon nanotubes

Andrew Presley: (25%) Preparation of recombinant TMV coat protein bearing reactive functional groups for bioconjugation